

Re-exposure to beta cell autoantigens in pancreatic allograft recipients with pre-existing beta cell autoantibodies.

Muhammad Ahmad Mujtaba¹, Jonathan Fridell⁶, Benita Book⁴, Sara Faiz², Asif Sharfuddin³, Eric Wiebke⁵, Mark Rigby⁴, Tim Taber³

University of Texas Medical Branch Galveston

Department of Medicine, Division of Nephrology/Transplant ¹

Department of Pathology²

Indiana University School of Medicine

Department of Medicine, Division of Nephrology/Transplant³

Transplant Immunology Lab ⁴

Pediatrics⁵

Division of Surgery ⁶

Corresponding Author:

Muhammad Ahmad Mujtaba MD, FASN, FAST

Email: mamujtab@utmb.EDU

Division of Nephrology, University of Texas Medical Branch Galveston, TX 77555

Phone 409-772-1811, Fax: 409-772-5451

Running Headline: β cell autoantigens in pancreas transplant

Word count excluding including abstract: 2312

This is the author's manuscript of the article published in final edited form as:

Mujtaba, M. A., Fridell, J., Book, B., Faiz, S., Sharfuddin, A., Wiebke, E., ... Taber, T. (2015). Re-exposure to beta cell autoantigens in pancreatic allograft recipients with preexisting beta cell autoantibodies. *Clinical Transplantation*, 29(11), 991–996. <http://doi.org/10.1111/ctr.12619>

Re-exposure to beta cell autoantigens in pancreatic allograft recipients with pre-existing beta cell autoantibodies.

Abstract:

Re-exposure to beta cell autoantigens and its relevance in the presence of donor specific antibodies (DSA) in pancreas allograft recipients is not well known.

Thirty-three patients requiring a pancreas transplant were enrolled in an IRB approved study.

They underwent prospective monitoring for DSA and beta cell autoantibody (BCAA) levels to GAD65, Insulinoma-associated-antigen 2(IA-2), insulin (micro-IAA [mIAA]), and islet-specific zinc-transporter isoform-8 (ZnT8).

Twenty-five (75.7%) had pre transplant BCAA. Twenty had a single antibody (mIAA n=15, GAD65 n=5); five had two or more BCAA (GAD65+mIAA n=2, GAD65+mIAA+IA-2 n=2, GA65+mIAA+IA-2+ZnT8=1). No changes in GAD65 ($p>0.29$), IA-2 (>0.16), and ZnT8 ($p>0.07$) were observed between pre-transplant and post-transplant at 6, or 12-months. A decrease in mIAA from pre to post 6-months ($p<0.0001$), 12-months ($p<0.0001$), and from post-6 to post 12-months ($p=0.0002$) was seen. No new BCAA was observed at 1-year. Seven (21.0%) developed denovo-DSA. The incidence of DSA was 24% in patients with BCAA vs. 25% in patients without BCAA ($p=0.69$). Pancreatic allograft function of patients with vs. without BCAA, and with and without BCAA+DSA was comparable until last follow-up (3yrs). Re-exposure to beta cell autoantigens by pancreas transplant may not lead to increased levels or development of new BCAA or pancreas allograft dysfunction.

Introduction:

Type 1 diabetes mellitus (T1DM) is a T cell mediated autoimmune disease in which the β cells of the islets of Langerhans are selectively destroyed (1). Autoantibodies to beta cell antigens are present at, and required for the, the diagnosis of T1D (2, 3). The timing and number of autoantibodies precedes and are predictive of clinical onset of disease (4). The exact mechanisms involved with the interactions of beta cell autoantigens and cells of the innate (macrophages and dendritic cells) and adaptive (T and B cells) regarding the initiation and progression of beta cell destruction are not clear (5-7). Beta cell autoantigens are processed by macrophages, dendritic cells, or B cells in the pancreatic islets and presented to auto reactive CD4⁺ T cells in the peripheral lymphoid system. These auto reactive CD4⁺ T cells are activated and secrete cytokines, which can activate β cell-specific cytotoxic CD8⁺ T cells. The activated T cells are recruited to the pancreatic islets and produce cytokines, which further activate macrophages and other T cells, contributing to the destruction of β cells(8). The first diabetes autoantibodies that were identified were islet cell autoantibodies (ICAs), which actually represent autoimmunity to several different antigens (1, 2). More recently, autoantibodies specific to single tissue antigens, termed biochemical autoantibodies, have been identified (9-14). These include antibodies to GAD 65(GAD65); the antibody to an Insulinoma associated Antigen-2 (ICA512), antibodies to insulin (IAA), and antibodies to islet-specific zinc transporter isoform 8 (ZnT8). In order to improve diabetes metabolic control, some patients receive a pancreas transplant or an islet cell transplant. In many cases this is done in conjunction with a kidney transplant due to diabetes-related renal failure. In most cases, this results in improved metabolic control and in some instances insulin independence. Yet even though patients are receiving powerful immune suppression for their transplants, some patients reject their pancreas or islet cell transplants while maintaining their renal graft. The rejection of their pancreas/islet cells appears to due to a combination of alloimmunity and recurrent autoimmunity. (5-7). Development or reemergence of autoantibodies to islet beta cells (BCAA) and denovo HLA donor specific alloantibodies (DSA) are considered to be important prognostic markers (15). We carried out the present study to analyze the trend of diabetes autoantibodies and HLA DSA antibodies in successful whole

organ pancreatic transplant recipients with our practice of induction and maintenance immunosuppression. Here we present our findings.

Methods:

Study Subjects: Thirty-three patients with previously diagnosed Type 1 diabetes with technically successful pancreas transplant (13 pancreas-alone [PTA], 20 with a kidney [SPK] were enrolled in a IRB approved clinical study. Serum samples were collected prospectively immediately before transplantation at 6 and 12 months and stored frozen until testing in batch analysis. All pancreas allografts were implanted with systemic venous and enteric exocrine drainage. Induction immunosuppression consisted of rabbit antithymocyte globulin (1 mg/kg x 5) and steroid withdrawal with the addition of anti-CD20 for PTA recipients. Routine maintenance immunosuppression included tacrolimus (12 hours trough 6-8ng/ml) and sirolimus (24 hour trough 4-6ng/ml) with the addition of mycophenolate mofetil (500 mg bid) for PTA. All subjects are alive with a functioning pancreas allograft at three years as assessed by clinical follow-up.

Anti-HLA Antibody Determination: Donor specific antibody specificity (DAS) was analyzed using LUMINEX platform solid phase based test systems (LabScreen®, One Lambda Inc., Canoga, CA). Microbeads coated with individual purified Class I or Class II HLA antigen are incubated with patient serum that has been pretreated with DTT to reduce IgM antibodies. Beads are then washed and labeled with R-Phycoerythrin (PE) conjugated goat anti-human IgG. Luminex acquisition data is analyzed to determine test pattern reactivity and assign specificity. Mean fluorescence intensity (MFI) greater than 2600 for Class I and greater than 3600 for Class II is considered positive based on correlation with flow cytometric crossmatches. Results are determined using manufacturer's Fusion software.

Diabetes-related Autoantibodies: All sera were tested at the Barbara Davis Center Aurora, CO. as follows.

GAD65 and IA-2 Autoantibody Standardized Radioassay: GAD65 autoantibody (GADA) and IA-2 autoantibody (IA-2A) radioassay were performed with NIDDK harmonized standard

methods. The GAD65 cDNA (full length), pThGAD65, was kindly provided by Dr. Åke Lernmark and IA-2 cDNA (intracellular domain, a.a. 605 to 979), IA-2ic, by Dr. Ezio Bonifacio). Labeled GAD65 and IA-2 proteins were produced by *in vitro* transcription and translation. *In vitro* translated [35S] GAD65 (20,000 cpm) or [35S] IA-2 (20,000 cpm) in an antigen buffer and incubated overnight at 4 °C with 2 µl of patient serum at a final 1:12.5 dilution. Autoantibody-bound antigen was precipitated with 25 µl of 50% protein A-Sepharose. Radioactivity was counted on a TopCount 96-well plate β-counter (Perkin Elmer). The results were calculated with an equation from the standard curve in each assay and expressed as DK units/ml.

Insulin autoantibody assay (mIAA): Insulin autoantibody (IAA) was measured by a micro-IAA assay (mIAA). Briefly, 125-I labeled human insulin (Perkin Elmer) was incubated with patient serum with and without cold human insulin and immune complex was precipitated with protein A and G Sepharose. The assay was performed on a 96-well filtration plate and radioactivity was counted on a Topcount 96-well plate beta counter. An index was calculated upon delta cpm between wells without and with cold insulin, with a positivity criterion of 0.010.

ZnT8 Autoantibody Radioassay: ZnT8 autoantibody (ZnT8A) was performed with a radioimmunoassay. The ZnT8 cDNA, ZnT8-RW dimmer (JH5.2), was kindly provided by Dr. John Hutton. The ZnT8 protein was produced by *in vitro* transcription and translation (Promega TNT kit) and labeled with [35S] methionine (Perkin Elmer). Labeled [35S] ZnT8 (20,000 cpm) was mixed and incubated overnight at 4 °C with patient serum at a final 1:25 dilution. Autoantibody-bound antigen was precipitated with 25 µl of 50% protein A-Sepharose. Radioactivity was counted on a TopCount 96-well plate β-counter (Perkin Elmer). The results are expressed as index (index = (sample CPM – negative control CPM)/ (positive control CPM – negative control CPM)).

Statistical Analysis:

Statistical analysis used Wilcoxon signed rank tests. A p value of <0.05 was considered significant.

Results:

The majority of patients were Caucasian (88%) and male (51%) with median age of 45 years and mean BMI of 25. Median duration of T1DM was 30 years and majority were SPKs. Median follow up on these patients is 36 months. GAD65, mIAA, IA-2 and diabetes autoantibodies were assessed in all patients from prospectively collected banked samples taken at baseline, and 6 and 12 months following transplant. Twenty-five (75.7%) had at least one diabetes auto-antibodies pre-transplant. No significant difference in the trough levels of tacrolimus, sirolimus was observed at specified time intervals. Fasting blood sugar levels, A1C, C-peptide and serum lipase levels were also recorded at specified intervals, no significant differences were observed in the patients with or without autoantibodies at the time of last follow up of 36 months

Of those patients with at least one diabetes autoantibody, 20 (80%) had one type of beta cell autoantibody (mIAA n=15, GAD65 n=5), whereas 2 or more beta cell autoantibody (GAD65+mIAA n=2, GAD65+mIAA+IA-2 n=2, GAD65+mIAA+IA-2+ZnT8=1) were detected in 5 (20%) patients. No statistically significant changes in GAD 65($p>0.29$), IA-2 (>0.16), and ZnT8 ($p>0.07$) were observed between pre-transplant and post-transplant antibody levels at 6, and 12 months [Table 1], however a significant decrease was noted in mIAA titers from pre to 6 months post-transplant ($p<0.0001$), from pre to post 12 months ($p<0.0001$), and from post 6 to post 12 months ($p\ 0.0002$). No new formation of beta cell autoantibody was observed at 1 year of follow up [Figure 1]. There was no significant difference at 6,12,24 and 36months in pancreas allograft function as assessed by clinical parameters of fasting blood sugar levels, A1C, C-peptide and serum lipase levels in patients with one autoantibody versus two or more autoantibodies.

Patients were also assessed for DSA during these times. One patient had pre-transplant DSA whereas 7 (21.0%) patients developed de novo DSA at median follow up of 76 days. Incidence of DSA was 24% (n=6) in patients with any diabetes autoantibody vs. 25% (n=2) in patients without autoantibodies ($p=0.69$). Seven patients had both class I and class II DSA. Mean peak class I DSA-MFI was 3529 (± 1456); class II DSA-MFI was 5412 (± 2204) whereas cumulative DSA MFI (CI + CII) was 8941 (± 3660). Figure 2 shows trend of DSA in individual patient. No

significant difference was noted in the DSA MFI of patients with or without beta cell autoantibodies. No significant difference was noted in the pancreatic allograft function parameter of patients with HLA DSA antibodies and autoantibodies vs HLA DSA antibodies without beta cell autoantibodies. All patients are insulin independent with detectable C-peptide levels and A1C of 5.4+/- 0.25 at last clinical follow-up of three years.

Discussion:

Type 1 diabetes is an autoimmune disorder leading to loss of pancreatic β -cells and insulin secretion, followed by exogenous insulin dependence. Daily insulin administration is required in those with T1DM for survival, yet it is not a cure. Those with T1DM continue to suffer significant morbidities and a higher mortality than those without T1DM (16). In an attempt to improve metabolic control and lessen the side effects of T1DM, some patients undergo whole pancreas or islet cell transplantation, but then require powerful immune suppressive medicine to maintain the graft. With improved immunosuppression and technical expertise, immediate failures of whole pancreas grafts have become less frequent; however chronic pancreatic allograft loss remains a concern (17). This chronic pancreatic allograft dysfunction may be related to development of HLA DSA or beta cell autoantibodies. Post-transplant DSA monitoring is now considered standard of care in pancreas transplantation at most centers. HLA DSA may not impact early pancreas allograft but have been reported to impair long-term outcomes (18, 19). Similarly, although rare, recurrence of beta cell autoimmunity has been reported in whole organ pancreas transplant (20). Our study is unique, as it looks at both the HLA donor specific antibodies as well as beta-cell autoantigens responses in whole pancreas recipients at defined time intervals. From the islet transplant experience there is growing evidence that recurrence of beta cell autoimmunity is a critical contributor to loss of the transplanted islets even in the face of potent immune suppression (15). Thus, markers that might assist in early identification of recurrent autoimmunity may assist in the choice of suitable candidates for islet cell transplantation or alternative immunomodulatory approaches. The absence of DSA in PTA recipients who have received rituximab following Thymoglobulin induction is a very interesting finding of our study. Along with this, we did not observe early recurrence of beta cell autoantibodies and the patients have excellent pancreatic allograft

function based upon clinical parameters of normoglycemia, A1C, c-peptide and pancreatic enzyme levels.

Recent data from islet cell transplant antibody monitoring by Lorenzo et al shows that presence or changes in DSA or Islet cell antibody predicts outcome in type DM1. In their study, the incidence of pre-islet cell transplant DSA and islet antibodies was 49% and 44% respectively. In 66% of these patients an increase in DSA and autoantibodies was detected on the follow up. The study also shows reduced islet survival in patients with increasing antibodies with a median time to graft loss of 318 days after donor specific antibodies (DSA) increase and 117 days after autoantibody increase (15). We observed that pancreas allograft behaves differently with regards to post-transplant beta cell autoantibodies and development of denovo donor specific antibodies compared to reported experience with islet transplant. This relatively weaker auto-immunity and absence of development of post-transplant donor specific antibodies in PAT with inclusion of rituximab as part of induction therapy requires further studies. Dr Pescovitz from our institution has previously demonstrated that rituximab partially preserved beta cell function over a period of 1 year in patients with type T1DM (21). In our study development of denovo DSA was not associated with the pre or post-transplant beta auto-antibody status. Also note that this group of patients are on a steroid free maintenance immunosuppression protocol.

The limitations of our reported data are lack of pancreatic allograft biopsy and relatively shorter follow up. Strengths include adequate number of patients with prospective data and to our knowledge the only study where DSA and beta cell autoantibodies were concomitantly evaluated.

From our reported data one can conclude that re-exposure to beta-cell autoantigens in pancreatic allograft recipients with pre-existing beta cell auto antibodies may not have a significant impact on short and medium term whole organ pancreas transplant outcomes. The incidence of denovo post-transplant beta cell autoantibodies also appears to be very low. Future studies with large number of patients and longer follow up are required to determine long term impact of re exposure to beta cell auto antibodies in pancreas transplant recipients.

Disclosure:

None related to this manuscript

References:

1. Eisenbarth GS, Srikanta S, Jackson R, Rabinowe S, Dolinar R, Aoki T, et al. Anti-thymocyte globulin and prednisone immunotherapy of recent onset type 1 diabetes mellitus. *Diabetes research*. 1985;2(6):271-6.
2. Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet*. 1974;2(7892):1279-83.
3. Lendrum R, Walker G, Cudworth AG, Theophanides C, Pyke DA, Bloom A, et al. Islet-cell antibodies in diabetes mellitus. *Lancet*. 1976;2(7998):1273-6.
4. Riley WJ, Maclaren NK, Krischer J, Spillar RP, Silverstein JH, Schatz DA, et al. A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. *The New England journal of medicine*. 1990;323(17):1167-72.
5. Tyden G, Reinholt FP, Sundkvist G, Bolinder J. Recurrence of autoimmune diabetes mellitus in recipients of cadaveric pancreatic grafts. *The New England journal of medicine*. 1996;335(12):860-3.
6. Sutherland DE, Goetz FC, Sibley RK. Recurrence of disease in pancreas transplants. *Diabetes*. 1989;38 Suppl 1:85-7.
7. Petruzzo P, Andreelli F, McGregor B, Lefrancois N, Dawahra M, Feitosa LC, et al. Evidence of recurrent type I diabetes following HLA-mismatched pancreas transplantation. *Diabetes & metabolism*. 2000;26(3):215-8.
8. Yoon JW, Jun HS. Autoimmune destruction of pancreatic beta cells. *American journal of therapeutics*. 2005;12(6):580-91.

9. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, et al. Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science*. 1983;222(4630):1337-9.
10. Christie MR, Vohra G, Champagne P, Daneman D, Delovitch TL. Distinct antibody specificities to a 64-kD islet cell antigen in type 1 diabetes as revealed by trypsin treatment. *The Journal of experimental medicine*. 1990;172(3):789-94.
11. Payton MA, Hawkes CJ, Christie MR. Relationship of the 37,000- and 40,000-M(r) tryptic fragments of islet antigens in insulin-dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2 (ICA512). *The Journal of clinical investigation*. 1995;96(3):1506-11.
12. Lampasona V, Buzzetti R, Bazzigaluppi E, Petrone A, Capizzi M, Tiberti C, et al. Zinc transporter 8 antibodies complement GAD and IA-2 antibodies in the identification of adult-onset autoimmune diabetes. *Diabetologia*. 2008;51:S237-S.
13. Achenbach P, Bonifacio E, Williams AJK, Ziegler AG, Gale EAM, Bingley PJ, et al. Autoantibodies to IA-2 beta improve diabetes risk assessment in high-risk relatives. *Diabetologia*. 2008;51(3):488-92.
14. Vaziri-Sani F, Oak S, Radtke J, Lernmark A, Lynch K, Agardh CD, et al. ZnT8 autoantibody titers in type 1 diabetes patients decline rapidly after clinical onset. *Autoimmunity*. 2010;43(8):598-606.
15. Piemonti L, Everly MJ, Maffi P, Scavini M, Poli F, Nano R, et al. Alloantibody and autoantibody monitoring predicts islet transplantation outcome in human type 1 diabetes. *Diabetes*. 2013;62(5):1656-64.
16. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *The New England journal of medicine*. 2005;353(25):2643-53.
17. Kandula P, Fridell J, Taber TE, Sharfuddin A, Yaqub MS, Phillips CL, et al. Impact of tacrolimus-sirolimus maintenance immunosuppression on proteinuria and kidney function in pancreas transplant alone recipients. *Transplantation*. 2012;94(9):940-6.
18. Mujtaba MA, Fridell JA, Higgins N, Sharfuddin AA, Yaqub MS, Kandula P, et al. Early findings of prospective anti-HLA donor specific antibodies monitoring study in pancreas transplantation: Indiana University Health Experience. *Clin Transplant*. 2012;26(5):E492-9.

19. Mittal S, Page SL, Friend PJ, Sharples EJ, Fuggle SV. De novo donor-specific HLA antibodies: biomarkers of pancreas transplant failure. *Am J Transplant*. 2014;14(7):1664-71.
20. Braghi S, Bonifacio E, Secchi A, Di Carlo V, Pozza G, Bosi E. Modulation of humoral islet autoimmunity by pancreas allotransplantation influences allograft outcome in patients with type 1 diabetes. *Diabetes*. 2000;49(2):218-24.
21. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Golan R, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *The New England journal of medicine*. 2009;361(22):2143-52.

Figure Ligands:**Figure 1:**

mIAA decreased significantly from pre to post1 ($p < 0.0001$), from pre to post2 ($p < 0.0001$), and from post1 to post2 ($p = 0.0002$). No statistically significant changes in GAD65 ($p > 0.29$), IA-2 ($p > 0.16$), or ZnT8 ($p > 0.07$). Note; Figures are Medians. p-value from Wilcoxon signed rank test

Figure 2:

HLA DSA monitoring, SPK; simultaneous pancreas and kidney transplant.

